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Synthesis and antimicrobial activity of imidazo- and pyrimido[2,1-*f*]-theophyllines

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Abstract Heating of 8-aminotheophylline with methyl (*Z*)-2-benzoylamino-3-(dimethylamino)propenoate in acetic acid afforded in a one-pot synthesis a new pyrimido[2,1-*f*]theophylline derivative. Methylation of this by using CH_3I/NaH furnished in good yield the double methylated derivative. Furthermore, glycosidation of the former with 1-α-bromo-2,3,4,6-tetra-*O*-acetyl-D-glucose gave the β-glucoside derivative. Reaction of 8-aminotheophylline with [bis(methylthio)methylene]malonitrile, ethyl[bis(methylthio)-methylene]cyanoacetate, 1,3-diphenylprop-2-en-1-one, 2-cyano-1,3-diphenylprop-2-en-1-one, 1-(4-nitrophenyl)-3-(dimethylamino)prop-2-ennitrile, 1-phenyl-3-(dimethylamino)prop-2-en-1-one, 2-substituted 3-aryl or heteroarylprop-2-ennitrile and ethyl(arylmethylene)cyanoacetate in *N*,*N*-dimethylformamide in the presence of anhydrous potassium carbonate afforded also the corresponding new derivatives of pyrimido-[2,1-*f*]theophylline. However, 8-aminotheophylline reacted in similar manner with 3-chloropentan-2,4-dione and 2-bromo-1-phenylethanone to give the corresponding imidazo[2,1-*f*]theophyllines. Furthermore, azo-coupling of one of these with 4-methylphenyldiazonium chloride was performed. The antimicrobial activity of the products has been evaluated. The structures of all new compounds obtained were established by their spectral analyses.

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Keywords Theophylline; Fused purines; Methylxanthines; Glycoside; Antimicrobial activity.

Introduction

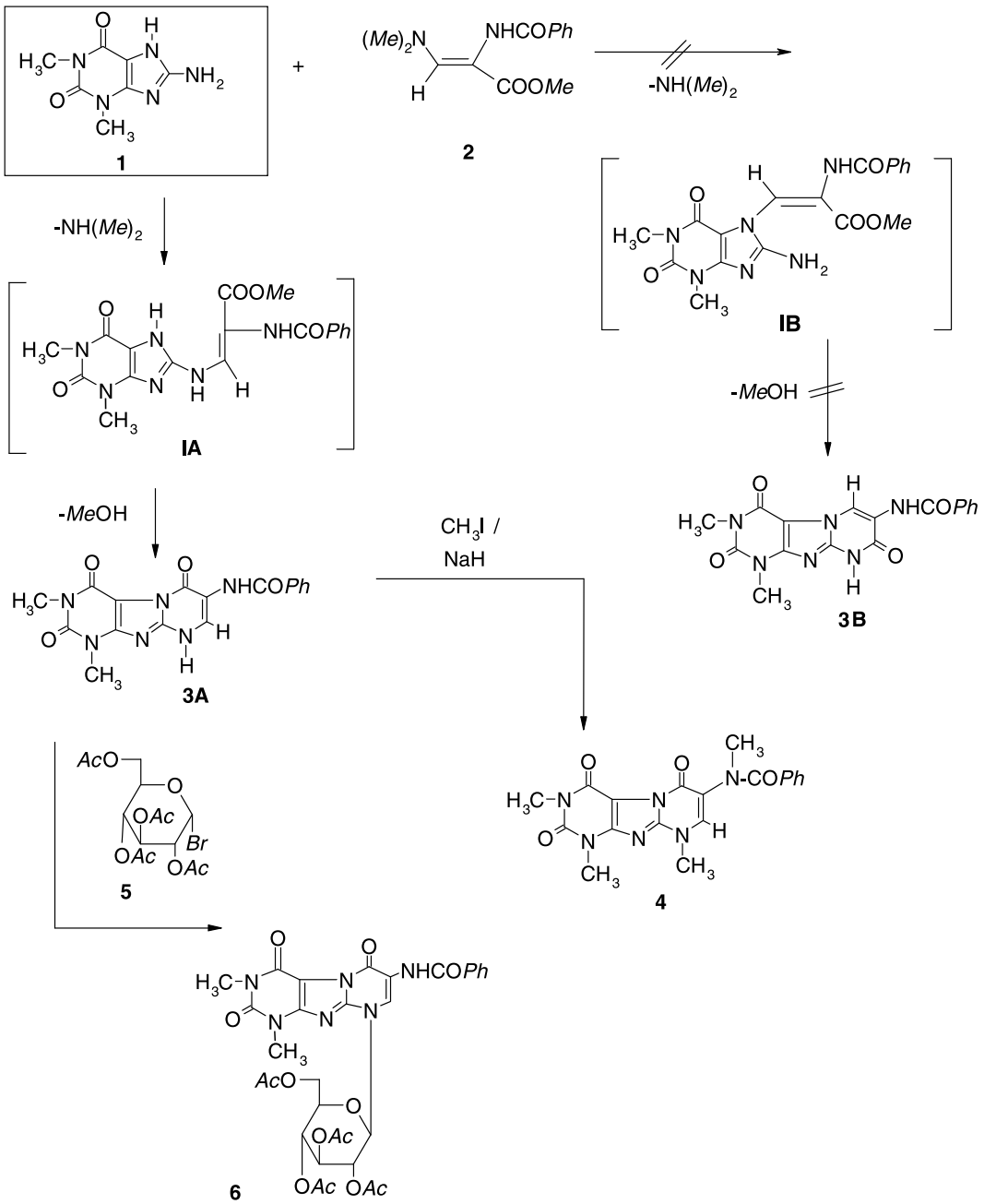
Among new alkylxanthines, 7- and 8-substituted derivatives were investigated in respect of their bronchospasmolytic [1–4], anticancer [5], and circulatory blood system activity [6]. A large amount of work has been performed on the fused systems derived from theophylline, including synthetic procedures and structure determination [7–15] but only few of the synthesized new heterocyclic derivatives were pharmacologically tested, which revealed antiinflammatory [16], anti P-388 leukemia [17], and vascular relaxing agents [18]. Recently, it has been found that anellation of a six or seven membered ring at the 7,8-positions of theophylline changed the profile of its CNS activity [19, 20].

In literature several examples of [*f*]-fused purines have been reported including pyrrolo[2,1-*f*] [21], oxazolo[2,3-*f*] [22, 23], imidazo[2,1-*f*] [24–26], pyrido[2,1-*f*] [21], pyrimido[2,1-*f*] [21, 27–32, 36], oxazino[2,3-*f*] [33], pyrazino[2,1-*f*] [21], diazepino[2,1-*f*] [20, 3], 2,4-benzodiazepino[3,2-*f*] [34], 1,2,4-triazino[3,2-*f*] [35–37], and 1,2,4-triazepino-[3,2-*f*] [35] purines. As part of our studies of new fused purine compounds as potential antimicrobial agents, we wish to report the synthesis of new derivatives of imidazo and pyrimido[2,1-*f*]purine *via*

reaction of 8-aminotheophylline with ketene, thioacetal, propenenitrile, and haloketone derivatives.

Results and discussion

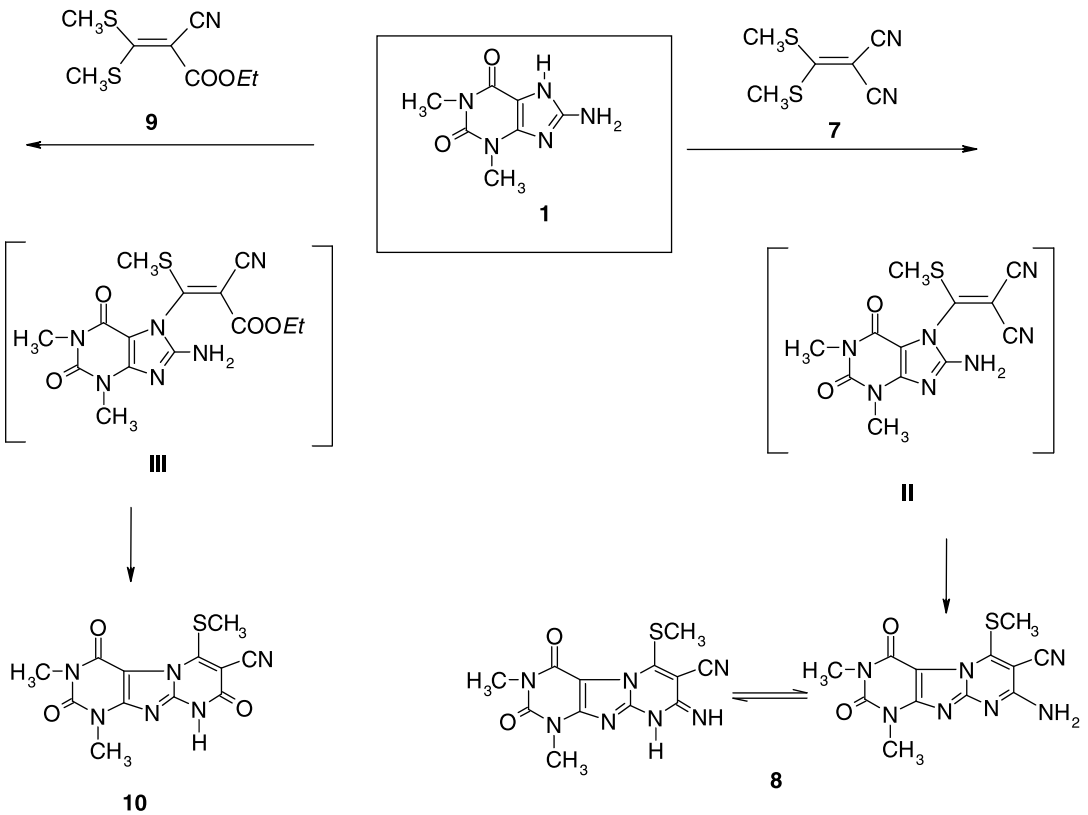
The starting 8-aminotheophylline (**1**) was prepared as previously reported [38]. Refluxing of **1** with methyl (*Z*)-2-benzoylamino-3-(dimethylamino)propenoate (**2**) in acetic acid for 15 h gave a single product as indicated by TLC analysis of the crude product. The structure of the isolated product was established on the basis of its spectral (MS, IR, and ^{1}H NMR) analyses. The mass spectrum of the product isolated revealed a molecular ion peak (m/z) at 366.34 of $C_{17}H_{14}N_6O_4$. Its infrared spectrum revealed one absorption band of NH at $\bar{\nu} = 3409\,\mathrm{cm}^{-1}$ and no band of NH_2. Also the ^{1}H NMR spectrum showed 2NH signals at $\delta = 9.8$ and 13.8 ppm. Furthermore, the



Scheme 1

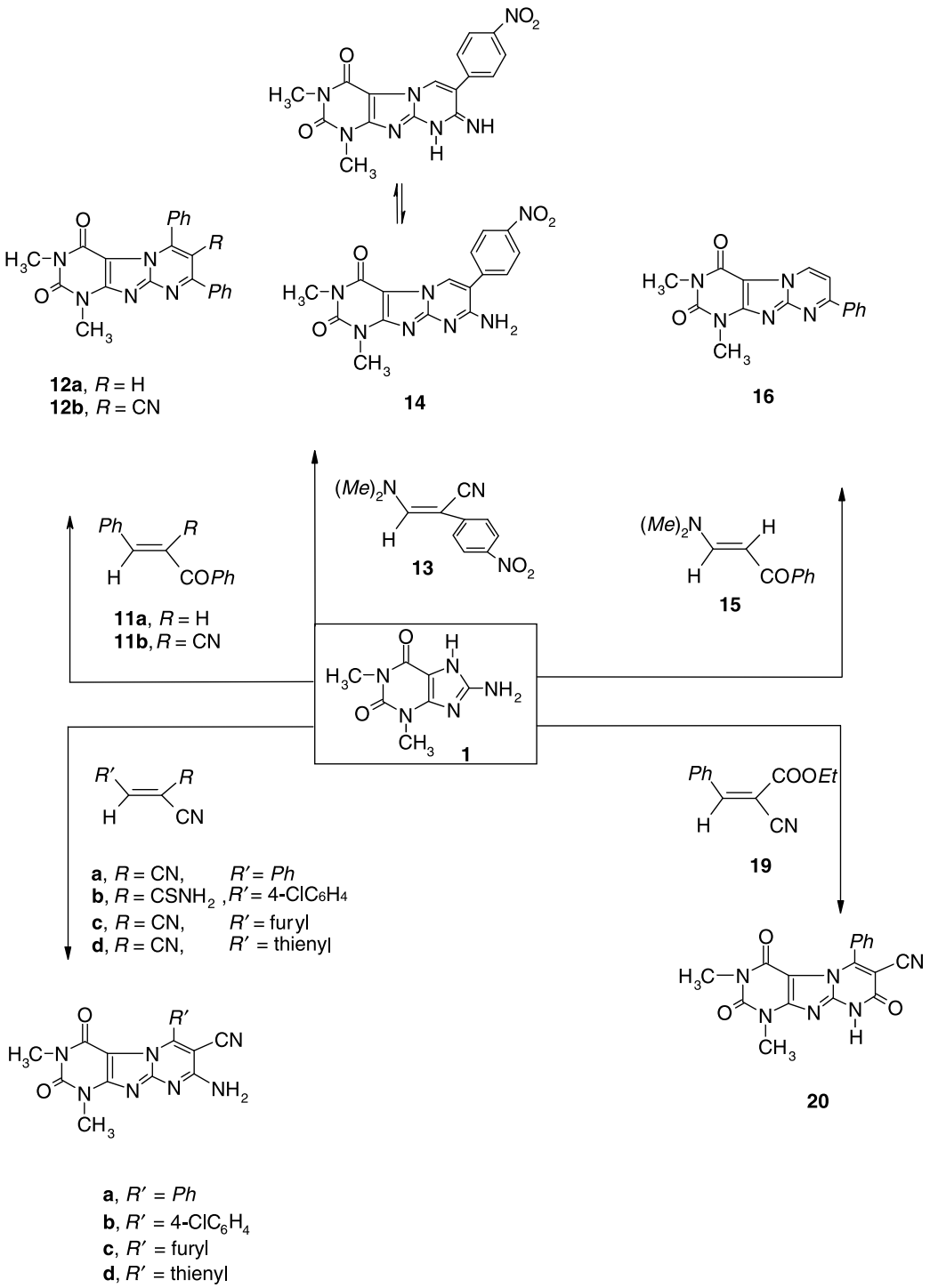
^{13}C NMR spectrum of the obtained product revealed 15 carbon signals. Since the chemical shift of the carbonyl carbon at position 6 of product **3** (168.8) is similar to that of the reported carbonyl carbon at position 6 (169) of 7-benzoylamino-1,3-diphenyl-pyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidin-5,6(1*H*,8*H*)-dione [39], these spectral data were in full agreement with the expected structure of the product obtained to resemble that of pyrimido[2,1-*f*]theophylline derivative **3A** and not the isomer **3B** (Scheme 1). Formation of **3A** might occur *via* initial electrophilic substitution of the 8-amino group of **1** in acidic medium [39, 47], to give **1A** as an intermediate which undergoes cyclization to the final product **3** (Scheme 1). The assignment of the structure **3A** is also substantiated by investigation of its methylation and glycosidation reactions. Thus, methylation of **3A** by using methyl iodide in the presence of sodium hydride yielded the double methylation product **4**. The ^{1}H NMR spectrum of the latter product **4** revealed two signals of new CH_3 groups at $\delta = 3.1$ (*Ph*CON–CH_3) and 3.15 (N9-CH_3) ppm. However, glycosidation of **3** with 1-α-bromo-2,3,4,6-tetraacetyl-D-glucose (**5**) afforded the glucoside derivative **6** (Scheme 1). The ^{1}H NMR spectrum of **6** showed the anomeric proton as a doublet at $\delta =$ 6.2 ppm with a spin–spin coupling constant (*J*) of 10.5 Hz corresponding to a diaxial orientation of H-1′ and H-2′ protons indicating the β-glucoside [45].

The reaction of **1** with a molar equivalent of 2-(bis-methylthiomethylene) malonitrile (**7**) and ethyl[bis(methylthio)methylene]cyanoacetate (**9**) in refluxing *N*,*N*-dimethylformamide (*DMF*) containing equivalent amounts of anhydrous potassium carbonate for 15 h (evidenced by TLC) afforded the corresponding cyclized products, pyrimido[2,1-*f*]theophylline derivatives **8** and **10** (Scheme 2). The structures of **8** and **10** we confirmed by spectral data. The ^{1}H NMR of the product **8** showed a signal for SCH_3 protons at $\delta = 2.6$ and two signals of NH at $\delta = 8.0$ and 9.6 ppm and no signal of NH_2 was observed. This finding indicates that the structure of the latter product **8** exists in imine form. The ^{1}H NMR of the product **10** showed one NH signal at $\delta = 9.6$ ppm and no signal of NH_2. Moreover, the IR spectra of **8** and **10** revealed bands characteristic for a CN group.



Scheme 2

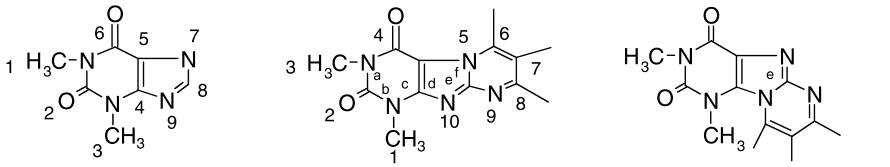
As reported in literature [21, 40], the formation of **8** and **10** may proceed *via* initial alkylation of the ring nitrogen in **1** to give **II** and **III**, respectively as intermediates which undergo cyclization to the final products **8** and **10**. The synthetic approach pointed out here was extended to enable the synthesis of other functionally substituted pyrimido-[2,1-*f*]theophyllines for their biological evaluation. When 8-aminotheophylline **1** was reacted with 1,3-diphenylprop-2-en-1-one (**11a**), 2-cyano-1,3-diphe-



Scheme 3

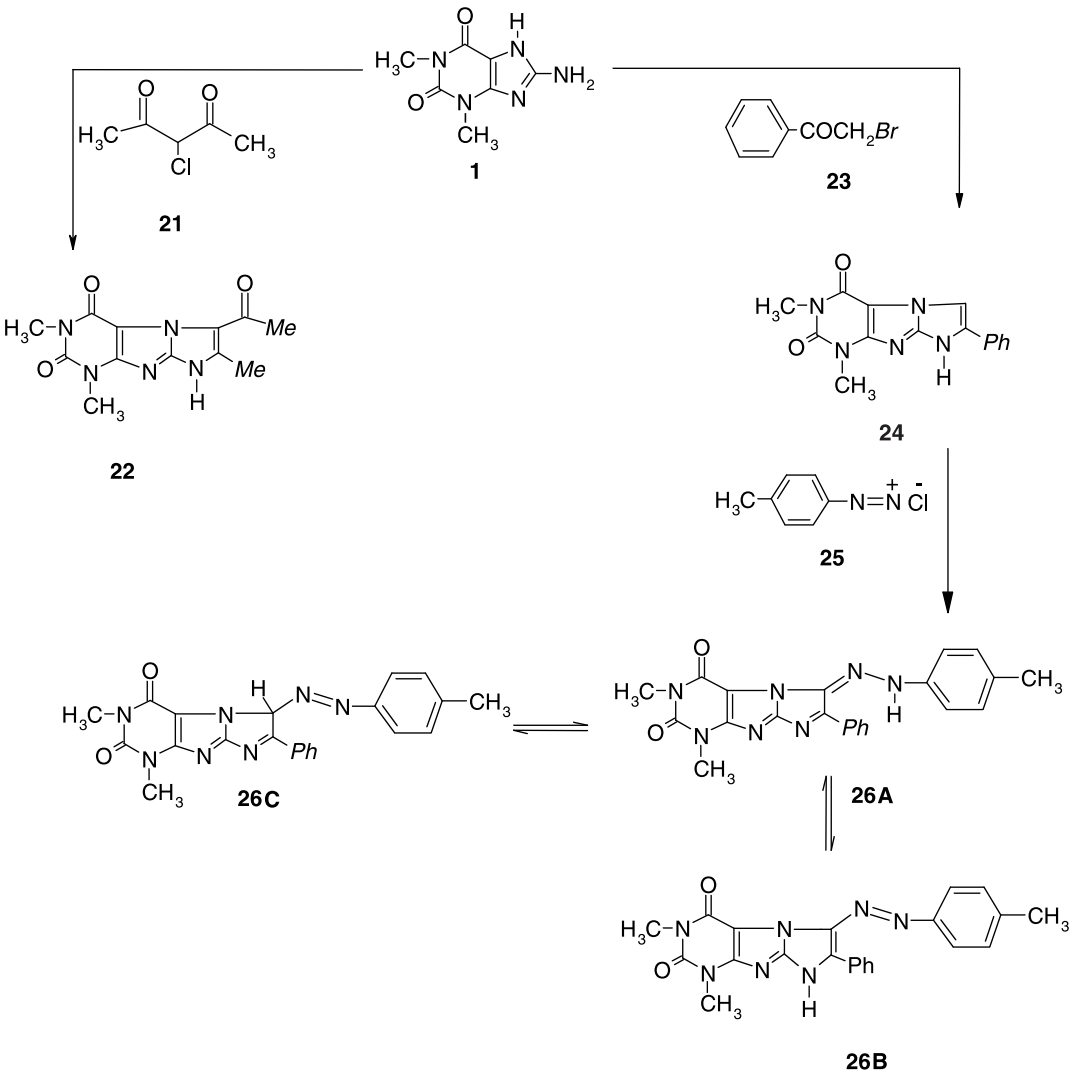
nylprop-2-en-1-one (**11b**), 1-(4-nitrophenyl)-3-dimethylaminoprop-2-ennitrile (**13**), and 1-phenyl-3-dimethylaminoprop-2-en-1-one (**15**) in refluxing *DMF* containing equivalent amounts of anhydrous potassium carbonate for 10 h (TLC), the corresponding pyrimido[2,1-*f*]theophylline derivatives **12a**, **12b**, **14**, and **16** were obtained (Scheme 3).

The structures of **12**, **14**, and **16** were established on the basis of their spectral data (MS, ^{1}H NMR, and IR). For example, the ^{1}H NMR spectrum of **12a**



Theophylline Pyrimido[*f*] theophylline Pyrimido[*e*]theophylline

Chart 1



Scheme 4

showed a characteristic signal at $\delta = 6.99$ ppm of the proton at position 7 and that of **14** revealed a signal at $\delta = 8.39$ ppm for the proton at position 6. Also in the ^{1}H NMR spectrum of product **16**, two doublet signals at $\delta = 7.54$ and 8.26 ppm with a coupling constant $J = 7.5$ Hz corresponding to CH=CH group of positions 6 and 7 are observed [19].

Similarly, by the reaction of **1** with 2-substituted 3-aryl or heteroarylprop-2-ennitrile **17** and ethyl-(arylmethylene)cyanoacetate **19** in refluxing *DMF* containing equivalent amounts of anhydrous potassium carbonate for 12 h, the corresponding pyrimido[2,1-*f*]theophylline derivatives **18** and **20** were obtained (Scheme 3). Elemental analyses and spectral data were consistent with the proposed structures of **18** and **19**. In the light of the foregoing results of all new pyrimidotheophylline derivatives **3**, **8**, **10**, **12**, **14**, **16**, **18**, and **20** obtained, it is proposed that all isolated products are consistent with a pyrimido[*f*]theophylline ring system and not the other isomeric pyrimido[e]theophylline (Chart 1) [19, 21, 46]. This is due to the steric hindrance caused by the proximity of the N1–CH_3 and substituents in the pyrimidine ring fused. In addition the ^{1}H NMR spectra of all isolated products revealed the signal of the N1–CH_3 protons at $\delta = 3.42$–3.65 ppm. This value is very close to that of N3–CH_3 of theophylline ($\delta = 3.59$ ppm) (Chart 1).

Attempts to prepare the ring system imidazo[2,1-*f*]theophylline were made by reacting 8-aminotheophylline (**1**) with 3-chloropentan-2,4-dione (**21**) and 2-bromo-1-phenylethanone (**23**) in refluxing *DMF* containing equivalent amounts of anhydrous potassium carbonate for 10 h. The corresponding imidazo-[2,1-*f*]theophyllines **22** and **24** were isolated, respectively (Scheme 4). The constitutions of the products **22** and **24** were confirmed by elemental and spectral analyses. In the ^{1}H NMR spectrum of **24**, a signal of aromatic CH at $\delta = 8.15$ ppm was observed. Treatment of **24** with 4-methylphenyl diazonium chloride (**25**) in ethanol containing sodium acetate at 0–5°C for 3 h afforded a single product **26** according to TLC. The structure of the latter product was elucidated by elemental analysis and spectral data. The IR spectrum revealed absorption bands of NH at $\bar{\nu} = 3451$ (NH) and two absorption bands of 2 CO at 1696 and 1643 cm^{-1}; its ^{1}H NMR showed a signal of NH proton at $\delta = 8.35$ ppm (D_2O exchangeable) and no signal of CH proton. Also the UV absorption spectrum of **26** in methanol revealed two absorption bands at λ_{max} 267 and 461 nm. These findings suggest that the isolated product **26** may be a mixture of hydrazone **26A** and azo tautomeric form **26B**, whereas the tautomeric form **26C** does not seem to play a role.

Antimicrobial activity

The compounds **3**, **4**, **8**, **10**, **12a**, **12b**, **16**, **18c**, **18d**, and **26** were evaluated for their antifungal and antibacterial activities against four fungal species namely *Aspergillus fumigatus* (*AF*), *Penicillium italicum* (*PI*), *Syncephalastrum racemosum* (*SR*), and *Candida albicans* (*CA*) as well as four bacteria species namely *Staphylococcus aureus* (*SA*), *Pseudomonas aeruginosa* (*PA*), *Bacillus subtilis* (*BS*), and *Escherichia coli* (*EC*).

The organisms were tested against the activity of solutions in a concentration of 1.0 $\mu g/cm^3$ of each compound and using inhibition zone diameter in cm (*IZD*) as a criterion for its antimicrobial activity.

Terbinafin as an antifungal agent and chloramphenicol as an antibacterial agent were used as references to evaluate the potency of the tested compounds under the same conditions. The results are depicted in Table 1. The results revealed that some compounds, such as **12a**, **12b**, **16**, **18c**, **18d**, and **26** have no activities against the tested organisms *PA*, *BS*, and *EC*, while compounds **12a**, **16**, and **26** exhibited the highest degree of inhibition against the tested organisms *SA* and *PI*.

Table 1 Antimicrobial activity of the products **3**, **4**, **8**, **10**, **12a**, **12b**, **16**, **18c**, **18d**, and **26***

Compound no.	*AF*	*PI*	*SR*	*CA*	*SA*	*PA*	*BS*	*EC*
3	0	0	0	0	+	0	+	0
4	+	0	0	+	0	0	0	0
8	0	0	0	0	+	0	+	+
10	0	0	+	0	+	0	+	0
12a	0	++	+	0	+	0	0	0
12b	0	+	0	0	+	0	0	0
16	0	+	0	0	++	0	0	0
18c	+	0	0	0	0	0	0	0
18d	0	0	0	0	+	0	0	0
26	0	++	0	+	+	0	0	0

*50 cm^3 of solution in *DMF* whose concentration was 1.0 $\mu g/cm^3$ was tested; chloramphenicol as standard antibacterial agent (*IZD* 1.0 cm); terbinfin as standard antifungal agent (*IZD* 1.0 cm); ++ *IZD* 0.6–1.0 cm; + *IZD* 0.1–0.5 cm; 0 no inhibition detected.

Experimental

IR spectra were determined on a KBr disc using a Perkin-Elmer 1650 (FT-IR) spectrophotometer, ^{1}H-NMR spectra were recorded on a Bruker AC 250 MHz and on a Varian Gemini 200 MHz NMR spectrometer using *TMS* as the internal reference; the mass spectra were recorded on a GC-MS spectrometer, the ionizing voltage was 70 eV. Thin layer chromatography was performed on silica gel sheets F 1550 LS 254 of Schleicher & Schüll. UV absorption spectra were recorded on a Perkin-Elmer Lambda 40 spectrophotometer. Melting points were measured on a Gallenkamp melting point apparatus. Elemental analyses were carried out at the Microanalytical Center of Cairo University and were within 0.4% of the theoretical values.

The starting materials such as 8-aminotheophylline [38] (**1**), methyl (*Z*)-2-benzoylamino-3-dimethylaminopropenoate [41] (**2**), [bis(methylthio)methylene]malonitrile [42] (**7**), ethyl[bis(methylthio)methylene]cyanoacetate [42] (**9**), 1,3-diphenylprop-2-en-1-one [43] (**11a**), 2-cyano-1,3-diphenylprop-2-en-1-one [43] (**11b**), 1-(4-nitrophenyl)-3-dimethylaminoprop-2-enenitrile [44] (**13**), 1-phenyl-3-dimethylaminoprop-2-en-1-one [44] (**15**), 2-substituted 3-aryl- or -heteroarylprop-2-enenitrile [43] (**17**), and ethyl (arylmethylene)cyanoacetate [43] (**19**) were prepared by literature methods. 3-Chloropentan-2,4-dione (**21**) and 2-bromo-1-phenylethanone (**23**) were bought from Aldrich.

7-Benzoylamino-1,3-dimethyl-pyrimido[2,1-f]purine-1,2,3,4,6,9-hexahydro 2,4,6-triones (**3**, $C_{17}H_{14}N_6O_4$)
A mixture of 3.90 g (0.02 mol) **1** and 4.96 g (0.02 mol) **2** in 50 cm^3 glacial acetic acid was heated under reflux for 15 h. The reaction was followed by TLC using $CHCl_3/CH_3OH$ (90/10, *v*/*v*) as eluent. The reaction solvent was evaporated *in vacuo* and the residue was recrystallized from *DMF*.

Yield 5.13 g (70%); $R_f = 0.22$; mp > 300°C; IR: $\bar{\nu} = 3409$ (NH), 1710, 1671, 1653, 1597 (4CO) cm^{-1}; ^{1}H NMR (*DMSO*-d_6, 200 MHz): $\delta = 3.2$ (s, 3H, N3–CH_3), 3.41 (s, 3H, N1–CH_3), 7.50–8.01 (m, 5H$_{arom}$), 8.3 (s, 1H, =CH), 9.8 (s, 1H, NH), 13.8 (s, 1H, NH) ppm; MS: *m*/*z* (%) = 366 (M$^+$, 30), 349 (5), 232 (5), 176 (3), 149 (35), 105 (100), 77 (40), 44 (25).

7-(Benzoylmethylamino)-1,3,9-trimethylpyrimido[2,1-f]-purine-1,2,3,4,6,9-hexahydro-2,4,6-trione (**4**, $C_{19}H_{18}N_6O_4$)
To a stirred suspension of 0.002 mol sodium hydride (60% oil) in 5 cm^3 *DMF*, a solution of 3.7 g (0.01 mol) **3** in 10 cm^3 *DMF* was added. The reaction mixture was cooled to 0–5°C and a solution of 0.0012 mol methyl iodide in 2 cm^3 *DMF* was added. The reaction mixture was stirred overnight and the solvent was evaporated. The residue was treated with 10 cm^3 ice-water and 2 cm^3 acetic acid and then stirred 2 h. The solid product was collected, washed with water, and recrystallized from dioxane/*DMF* (1/1, *v*/*v*) to give pure colourless powder [TLC using $CHCl_3/CH_3OH$ (90/10, *v*/*v*) as eluent].

Yield 0.3 g (75%); $R_f = 0.30$; mp > 300°C; IR: $\bar{\nu} = 1715$, 1680, 1650, 1600 (4CO) cm^{-1}; ^{1}H NMR (*DMSO*-d_6, 200 MHz): $\delta = 3.1$ (s, 3H, *Ph*CON–CH_3), 3.15 (s, 3H, N-9 CH_3), 3.25 (s, 3H, N3–CH_3), 3.65 (s, 3H, N1–CH_3), 7.2–7.5 (m, 5H$_{arom}$), 8.2 (s, 1H, =CH) ppm; MS: *m*/*z* (%) = 394 (M$^+$, 50), 366 (15), 289 (80), 261 (5), 233 (5), 204 (10), 176 (15), 135 (15), 106 (25), 96 (15), 77 (100), 67 (30), 51 (28), 42 (40).

7-Benzoylamino-1,3-dimethyl-9-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)pyrimido[2,1-f]purine-1,2,3,4,6,9-hexahydro-2,4,6-trione (**6**, $C_{31}H_{32}N_6O_{13}$)
To a solution of 3.7 g (0.01 mol) **3** in 0.01 mol aqueous potassium hydroxide in 6 cm^3 distilled water, a solution of 4.15 g (0.011 mol) **5** was added. The mixture was stirred at room temperature until the reaction was judged complete by TLC [using $CHCl_3/CH_3OH$ (90/10, *v*/*v*) as eluent, 15 h]. The mixture was evaporated under reduced pressure at 40°C and the residue was washed with distilled water to remove KBr. The product was filtered off, dried, and crystallized from dioxane.

Yield 5.6 g (80%); $R_f = 0.42$; mp 280–282°C; IR: $\bar{\nu} = 3240$ (NH), 1750, 1710, 1666, 1590 (4CO) cm^{-1}; ^{1}H NMR (*DMSO*-d_6, 200 MHz): $\delta = 1.90$–2.02 (4s, 12H, 4$COCH_3$), 3.35 (s, 3H, N3–CH_3), 3.65 (s, 3H, N1–CH_3), 4.05 (m, 2H, 6′, 6″–CH_2), 4.36 (m, 1H, 5′-H), 5.10 (t, 1H, 4′-H), 5.30 (t, $J = 9$ Hz, 1H, 2′-H), 5.70 (t, 1H, 3′-H), 6.20 (d, 1H, $J_{1',2'} = 10.5$ Hz, 1′-H), 7.2–7.5 (m, 5H$_{arom}$), 8.4 (s, 1H, =CH), 9.2 (s, 1H, NH) ppm.

1,3-Dimethylpyrimido[2,1-f]purine-1,2,3,4-tetrahydro-2,4-dione derivatives (general procedure)
Compound **1** (0.2 g, 0.001 mol) was dissolved in 50 cm^3 dry *DMF* by heating, 1.4 g (0.015 mol) anhydrous potassium carbonate were added, followed by addition of 0.001 mol **7**, **9**, **11a**, **11b**, **15**, **17**, **19**, **21**, or **23**. After being stirred under reflux for 10–15 h (TLC using ethyl acetate as eluent), the reaction mixture was concentrated *in vacuo*, poured into ice water, and neutralized with dilute HCl. The solid product precipitated, which was collected by filtration and recrystallized from the appropriate solvent.

8-Amino-1,3-dimethyl-6-(methylthio)-2,4-dioxo-1,2,3,4,8,9-hexahydropyrimido[2,1-f]purine-7-carbonitrile
(**8**, $C_{12}H_{11}N_7O_2S$)
Reflux 15 h; yield 0.22 g (70%); $R_f = 0.19$; mp > 300°C (*DMF*); IR: $\bar{\nu} = 3364$, 3274 (2NH), 2225 (CN), 1697, 1639 (2CO) cm^{-1}; ^{1}H NMR (*DMSO*-d_6, 200 MHz): $\delta = 2.6$ (s, 3H, SCH_3), 3.25 (s, 3H, N3–CH_3), 3.45 (s, 3H, N1–CH_3), 8.0 (s, 1H, NH), 9.6 (s, 1H, NH) ppm; MS: *m*/*z* (%) = 317 (M$^+$, 100), 300 (10), 284 (10), 231 (20), 205 (5), 180 (3), 165 (30), 109 (25), 94 (10), 82 (35), 67 (35), 42 (35).

1,3-Dimethyl-6-(methylthio)-2,4,8-trioxo-1,2,3,4,8,9-hexahydropyrimido[2,1-f]purine-7-carbonitrile (**10**, $C_{12}H_{10}N_6O_3S$)
Reflux 15 h; yield 0.21 g (65%); $R_f = 0.23$; mp > 300°C (*DMF*); IR: $\bar{\nu} = 3448$ (OH), 2217 (CN), 1705, 1655 (2CO) cm^{-1}; ^{1}H NMR (*DMSO*-d_6, 200 MHz): $\delta = 2.7$ (s, 3H, SCH_3) 3.20 (s, 3H, N3–CH_3), 3.50 (s, 3H, N1–CH_3), 9.6 (s, 1H, NH) ppm; MS: *m*/*z* (%) = 318 (M$^+$, 40), 279 (10), 261 (15), 192 (10), 135 (5), 105 (15), 77 (20), 44 (100).

1,3-Dimethyl-6,8-diphenylpyrimido[2,1-f]purine-2,4(1H,3H)-dione (**12a**, $C_{22}H_{17}N_5O_2$)
Reflux 10 h; yield 0.25 g (65%); $R_f = 0.22$; mp > 300°C (*Et*OH); IR: $\bar{\nu} = 1702$, 1663 (2CO) cm^{-1}; ^{1}H NMR (*DMSO*-d_6,

200 MHz): $\delta = 3.25$ (s, 3H, N3–CH_3), 3.57 (s, 3H, N1–CH_3), 6.99 (s, 1H, 7-CH=), 7.47–8.37 (m, 10H_{arom}) ppm; MS: m/z (%) = 384 ($M^+ + 1$, 30), 383 (M^+, 100), 325 (51), 248 (62), 297 (24), 221 (12), 216 (15), 142 (22), 105 (79), 77 (59), 56 (13).

1,3-Dimethyl-2,4-dioxo-6,8-diphenyl-1,2,3,4-tetrahydropyrimido[2,1-f]purine-7-carbonitrile (**12b**, $C_{23}H_{16}N_6O_2$)
Reflux 10 h; yield 0.22 g (55%); $R_f = 0.25$; mp > 300°C (*Et*OH); IR: $\bar{\nu} = 2192$ (CN), 1699, 1652 (2CO) cm^{-1}; ^1H NMR (*DMSO*-d_6, 200 MHz): $\delta = 3.38$ (s, 3H, N3–CH_3), 3.45 (s, 3H, N1–CH_3), 7.52–8.07 (m, 10H_{arom}) ppm; MS: m/z (%) = 408 (M^+, 23), 301 (10), 300 (19), 299 (100), 82 (38), 68 (21), 55 (24).

8-Amino-1,3-dimethyl-7-(4-nitrophenyl)pyrimido[2,1-f]-purine-2,4(1H,3H)-dione (**14**, $C_{16}H_{13}N_7O_4$)
Reflux 10 h; yield 0.26 g (70%); $R_f = 0.21$; mp > 300°C (Dioxane/*DMF*); IR: $\bar{\nu} = 3360$, 3341 (2 NH), 1703, 1640 (2CO) cm^{-1}; ^1H NMR (*DMSO*-d_6, 200 MHz): $\delta = 3.48$ (s, 3H, N3–CH_3), 3.52 (s, 3H, N1–CH_3), 7.35–8.35 (m, 5H_{arom}), 8.39 (s, 1H, 6-CH=), 9.2, 11 (s, 2H, NH_2) ppm; MS: m/z (%) = 367 (M^+, 29), 366 (17), 306 (22), 234 (15), 194 (13), 179 (15), 152 (18), 127 (22), 104 (17), 99 (28), 82 (20), 59 (44).

1,3-Dimethyl-8-phenylpyrimido[2,1-f]purine-2,4(1H,3H)-dione (**16**, $C_{16}H_{13}N_5O_2$)
Reflux 10 h; yield 0.18 g (60%); $R_f = 0.26$; mp > 300°C (*Et*OH); IR: $\bar{\nu} = 1703$, 1659 (2CO) cm^{-1}; ^1H NMR (*DMSO*-d_6, 200 MHz): $\delta = 3.24$ (s, 3H, N3–CH_3), 3.44 (s, 3H, N1–CH_3), 7.54 (d, $J = 7$ Hz, 1H, 7-CH=), 7.57–7.87 (m, 5H_{arom}), 8.26 (d, $J = 7$ Hz, 1H, 6-CH=) ppm; MS: m/z (%) = 307 (M^+, 66), 183 (33), 147 (33), 121 (53), 104 (100), 90 (53), 67 (35).

8-Amino-1,3-dimethyl-2,4-dioxo-6-phenyl-1,2,3,4-tetrahydropyrimido[2,1-f]purine-7-carbonitrile (**18a**, $C_{17}H_{13}N_7O_2$)
Reflux 12 h; yield 0.19 g (55%); $R_f = 0.19$; mp > 300°C (*Et*OH); IR: $\bar{\nu} = 3319$, 3260 (NH_2), 1704, 1637 (2CO) cm^{-1}; ^1H NMR (*DMSO*-d_6, 200 MHz): $\delta = 3.38$ (s, 3H, N3–CH_3), 3.57 (s, 3H, N1–CH_3), 7.50–7.89 (m, 5H_{arom}), 8.36 (br, 2H, NH_2) ppm; MS: m/z (%) = 347 (M^+, 25), 323 (32), 281 (33), 261 (29), 206 (62), 195 (49), 180 (18), 153 (27), 141 (8), 127 (32), 105 (11), 103 (23), 93 (30), 77 (78), 67 (67), 6 (31), 53 (49).

6-(4-Chlorophenyl)-1,3-dimethyl-2,4,8-trioxo-1,2,3,4,8,9-hexahydropyrimido[2,1-f]purine-7-carbonitrile
(**18b**, $C_{17}H_{12}ClN_7O_2$)
Reflux 12 h; yield 0.19 g (50%); $R_f = 0.18$; mp > 300°C; IR: $\bar{\nu} = 3320$, 3250 (NH_2), 1700, 1640 (2CO) cm^{-1}; ^1H NMR (*DMSO*-d_6, 200 MHz): $\delta = 3.37$ (s, 3H, N3–CH_3), 3.42 (s, 3H, N1–CH_3), 7.50–7.88 (m, 4H_{arom}), 8.30 (br s, 2H, NH_2) ppm; MS: m/z (%) = 383 (M^++1, 29), 382 (M^+, 41), 344 (41), 258 (79), 238 (73), 237 (85), 186 (41), 177 (38), 161 (100), 141 (52), 139 (47), 138 (61), 114 (38), 108 (35), 84 (67), 69 (61), 52 (44).

8-Amino-6-(2-furyl)-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimido[2,1-f]purine-7-carbonitrile (**18c**, $C_{15}H_{11}N_7O_3$)
Reflux 12 h; yield 0.14 g (42%); $R_f = 0.21$; mp > 300°C (*DMF*); IR: $\bar{\nu} = 3396$, 3210 (NH_2), 2201 (CN), 1698, 1649 (2CO) cm^{-1}; ^1H NMR (*DMSO*-d_6, 200 MHz): $\delta = 3.47$ (s, 3H, N3–CH_3), 3.51 (s, 3H, N1–CH_3), 7.10–7.70 (m, 3H, furan-H), 8.60 (br s, 2H, NH_2) ppm; MS: m/z (%) = 337 (M^+,75), 256 (80), 249 (60), 210 (75), 199 (100), 140 (60), 128 (50), 121 (70), 110 (75), 108 (80), 86 (100), 83 (70), 68 (50), 56 (65).

8-Amino-1,3-dimethyl-2,4-dioxo-6-(2-thienyl)-1,2,3,4-tetrahydropyrimido[2,1-f]purine-7-carbonitrile
(**18d**, $C_{15}H_{11}N_7O_2S$)
Reflux 12 h; yield 0.14 g (40%); $R_f = 0.20$; mp > 300°C (*DMF*); IR: $\bar{\nu} = 3326$, 3205 (NH_2), 2210 (CN), 1700, 1646 (2CO) cm^{-1}; ^1H NMR (*DMSO*-d_6, 200 MHz): $\delta = 3.37$ (s, 3H, N3–CH_3), 3.43 (s, 3H, N1–CH_3), 7.00–7.40 (m, 3H, thiophene-H), 8.00 (br s, 2H, NH_2) ppm; MS: m/z (%) = 354 (M^++1, 9), 353 (M^+, 5), 325 (30), 215 (7), 121 (7), 95 (11), 94 (100), 83 (10), 73 (9), 66 (10).

1,3-Dimethyl-2,4,8-trioxo-6-phenyl-1,2,3,4,8,9-hexahydropyrimido[2,1-f]purine-7-carbonitrile (**20**, $C_{17}H_{12}N_6O_3$)
Reflux 12 h; yield 0.14 g (40%); $R_f = 0.25$; mp > 300°C (*Et*OH); IR: $\bar{\nu} = 3334$ (NH), 2203 (CN), 1703, 1654 (2CO) cm^{-1}; ^1H NMR (*DMSO*-d_6, 200 MHz): $\delta = 3.34$ (s, 3H, N3–CH_3), 3.45 (s, 3H, N1–CH_3), 7.16–8.00 (m, 5H_{arom}), 8.10 (s, 1H, NH) ppm; MS: m/z (%) = 348 (M^+, 23), 275 (25), 195 (84), 176 (4), 138 (14), 105 (24), 91 (23), 82 (20), 77 (19), 68 (15), 52 (8).

6-Acetyl-1,3,7-trimethyl-1H-imidazo[2,1-f]purine-2,4(3H,8H)-dione (**22**, $C_{12}H_{13}N_5O_3$)
Reflux 10 h; yield 0.18 g (65%); $R_f = 0.29$; mp > 300°C (*DMF*); IR: $\bar{\nu} = 3419$ (NH), 1700, 1652 (2CO) cm^{-1}; ^1H NMR (*DMSO*-d_6, 200 MHz): $\delta = 2.27$ (s, 3H, CH_3), 2.44 (s, 3H, $COCH_3$), 3.17 (s, 3H, N3–CH_3), 3.47 (s, 3H, N1–CH_3), 8.71 (s, 1H, NH) ppm; MS: m/z (%) = 275 (M^+, 92), 255 (52), 201 (68), 190 (48), 167 (60), 150 (72), 112 (60), 109 (60), 100 (44), 90 (80), 80 (48), 67 (100), 60 (60).

1,3-Dimethyl-7-phenyl-1H-imidazo[2,1-f]purine-2,4(3H,8H)-dione (**24** $C_{15}H_{13}N_5O_2$)
Reflux 10 h; yield 0.18 g (60%); $R_f = 0.25$; mp > 300°C (*Et*OH); IR: $\bar{\nu} = 3395$ (NH), 1698, 1644 (2CO) cm^{-1}; ^1H NMR (*DMSO*-d_6, 200 MHz): $\delta = 3.40$ (s, 3H, N3–CH_3), 3.46 (s, 3H, N1–CH3), 7.31–8.08 (m, 5H_{arom}), 8.15 (s, 1H, CH=), 12.03 (s, 1H, NH) ppm; MS: m/z (%) = 295 (M^+,18), 295 (9), 238 (4), 209 (4), 105 (100), 77 (61), 67 (5), 51 (20).

(6E)-1,3-Dimethyl-7-phenyl-1H-imidazo[2,1-f]purine-2,4,6(3H)-trione 6-[(4-methylphenyl)hydrazone]
(**26**, $C_{22}H_{19}N_7O_2$)
A solution of 2.95 g (0.01 mol) **24** in 50 cm^3 ethanol was stirred with 1.4 g (0.01 mol) sodium acetate trihydrate for 15 min. The mixture was chilled in an ice bath at 0°C. While the solution was cooling, the 4-methylbenzene diazoni-

um chloride was prepared by the diazotization of 1.1 g (0.01 mol) p-toluidine in 6 cm^3 6 *M* hydrochloric acid with 10 cm^3 cold 1 *M* sodium nitrite solution in the usual way keeping the temperature below 5°C. The diazonium chloride solution was added to the reaction solution dropwise under stirring. The reaction mixture was left for 3 h in a refrigerator. The precipitated solid was filtered off, washed with water and ethanol, and dried. The product was recrystallized from 1,4-dioxane to give **26** as pure pale yellow crysrtals (TLC using ethyl acetate as eluent). Yield 0.29 g (70%); $R_f = 0.19$; mp > 300°C; IR: $\bar{\nu} = 3451$ (NH), 1696, 1643 (2CO) cm^{-1}; ^1H NMR (*DMSO*-d_6, 200 MHz): $\delta = 2.60$ (s, 3H, CH_3), 3.43 (s, 3H, N3–CH_3), 3.50 (s, 3H, N1–CH_3), 7.30–8.15 (m, 9H_{arom}), 8.35 (br s, 1H, NH) ppm; MS: m/z (%) = 414 (M^+, 80), 413 (100), 307 (11), 282 (35), 234 (3), 207 (3), 195 (3), 106 (26), 94 (10), 77 (25), 67 (45), 53 (9); UV (methanol): $\lambda_{max}(\varepsilon) =$ 267 (18200), 461 (9800) nm ($mol^{-1}\,cm^{-1}$).

Antimicrobial assay

Cultures of four fungal species *namely Aspergillus fumigatus* (*AF*), *Penicillium italicum* (*PI*), *Syncephalastrum racemosum* (*SR*), and *Candida albicans* (*CA*) as well as four bacterial species namely *Staphylococcus aureus* (*SA*), *Pseudomonas aeruginosa* (*PA*), *Bacillus subtilis* (*BS*), and *Escherichia coli* (*EC*) were used to investigate the antimicrobial activity of the compounds **3**, **4**, **8**, **10**, **12a**, **12b**, **16**, **18c**, **18d**, and **26**. The antimicrobial activity was assayed biologically using the diffusion plate technique. The latter technique was carried out by pouring a spore suspension of the fungal species (1 cm^3 of sterile water contains approximately 108 conidia) or spreading bacterial suspension over a solidified malt agar medium. The layer is allowed to set for 30 min. A solution of the test compounds (1.0 g/cm^3) in *DMF* was placed onto sterile 5 mm filter paper discs and allowed to dry, then the discs were placed on the centre of the malt agar plate and incubated at optimum incubation temperature 28 ± 2°C. The fungicide Terbinfin and the bactericide chloramphenicol were used as standards under the same conditions. Measurements were considered after 72 h for fungi and 24 h for bacteria. The results are summarized in Table 1.

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